

sensor. A reduced sensitivity of effector Th-cells towards oxidation is due to upregulation of Orai3 and of cytosolic antioxidants. The differential redox regulation of ORAI channels is a novel mechanism to tune Th-cell based immune responses during clonal expansion and inflammation.

1109-Plat

Comparative Analysis of Cholesterol Sensitivity of Kir Channels: Role of the Cytoplasmic Domain

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Kir channels are important in setting the resting membrane potential and modulating membrane excitability. A common feature of Kir2 channels and several other ion channels that has emerged in recent years is that they are regulated by cholesterol, a major lipid component of the plasma membrane whose excess is associated with multiple pathological conditions. Yet, the mechanism by which cholesterol affects channel function is not clear.

Here we show that in addition to Kir2 channels, members of other Kir subfamilies are also regulated by cholesterol. Interestingly, while similarly to Kir2 channels, several Kir channels are suppressed by an increase in membrane cholesterol, the function of others is enhanced following cholesterol enrichment. Furthermore, similarly to Kir2.1, and independent of the impact of cholesterol on channel function, we find that mutation of residues in the CD loop affect cholesterol sensitivity of Kir channels.

Among Kir2.1 CD loop residues, we have recently shown that the L222I mutation has the strongest effect on cholesterol sensitivity. This result is surprising since Kir2.2, which is as cholesterol sensitive as Kir2.1, already has an isoleucine at the corresponding position. Here we obtain further insight regarding the role of the cytosolic domain of Kir2 channels by examining mutations in adjacent cytosolic regions that also lead to loss of cholesterol sensitivity. In addition, we trace the source of the difference between Kir2.1 and Kir2.2 to a residue in the EF loop, N251, whose mutation to an aspartate reverses the effect of the L222I residue, and restores cholesterol sensitivity.

These findings suggest an indirect role of the cytosolic domain of Kir channels in regulating the effect of cholesterol on channel function and provide insight into the structural determinants of their gating mechanism.

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Molecular Mapping of An I_{Ks} Channel Opener Reveals Crucial Interactions Between KCNE1 and the Kv7.1 Voltage Sensor Paddle

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Voltage-gated K⁺ channels co-assemble with accessory subunits to form macromolecular complexes. In heart, assembly of Kv7.1 pore-forming subunits with KCNE1 auxiliary subunits generates the repolarizing K⁺ current I_{Ks}. We and others, recently suggested a strategic location of KCNE1 wedged close to helices S1 and S4 of two adjacent Kv7.1 voltage sensing domains (VSD) and nearby helix S6 of another Kv7.1 subunit. Here we show that the I_{Ks} channel opener, diisothiocyanostilbene-2',2''-disulfonic acid (DIDS) acts on I_{Ks} as a gating-modifier, thereby converting the time- and voltage-dependent channels into almost voltage- and time-independent currents. While DIDS activates Kv7.1, it does not affect Kv7.2. The two isothiocyanate functionalities are crucial for the potent activating effect of DIDS on I_{Ks}, since 4'-acetamido-4'-isothiocyanostilbene-2',2''-disulfonic acid (SITS) that has only one of these groups and 4,4'-dinitrostilbene-2,2''-disulfonic acid (DNDS), which lacks isothiocyanate groups and thus cannot form covalent bonds with amino acids, do not activate I_{Ks} currents. Mutagenesis and modeling data indicate that DIDS activates I_{Ks} by docking to an externally-accessible pocket, formed at the interface between the superficial N-terminal boundary of the KCNE1 transmembrane segment and the VSD paddle motif of Kv7.1. DIDS does not activate the channel complex formed by co-expression of KCNE1 and a chimeric Kv7.1 endowed with a Kv7.2 VSD paddle. DIDS binding at the Kv7.1 VSD-KCNE1 interface reveals that two lysine residues, K41 in KCNE1 and K218 in Kv7.1 S3-S4 linker are distant to about 10 [[Unable to Display Character: Å]]. Thus, KCNE1 affects Kv7.1 channel gating by closely interacting with the VSD paddle motif.

1111-Plat

Kv Channel Modulation: Closed State Block of Benzocaine But Not of Bupivacaine

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Local anaesthetics (LAs) block action potentials mainly by blocking Na channels. They are generally assumed to preferentially bind to channels in inacti-

vated and/or open state. Recently it has been suggested that they mainly bind to channels in intermediate closed states. This is based on the finding that LAs affect the currents time and voltage-dependently in voltage clamped channels; that they reduce the peak current more at low voltage steps than at high.

In previous studies on inactivating K channels we have concluded that LAs preferentially bind to channels in open state. In the present study we have re-analysed the effects of LAs on K channels with special reference to the new findings of closed state binding. We analysed the effects of bupivacaine and benzocaine on Kv3.1 and Shaker channels expressed in *Xenopus* oocytes. As shown previously bupivacaine induces a peaked current in both channel types. In accordance with the results on the Na currents bupivacaine reduced the peak less at +60 mV than at lower potentials. Nevertheless, a modelling analysis suggested that the results are explained by binding preferentially to open channels. In contrast, benzocaine did not induce a peak at any potential, but the early current was reduced more at low potentials than at high. The modelling analysis suggested that the effect is caused by binding to closed and open channels.

We thus conclude that bupivacaine and benzocaine blocks K channels differently; bupivacaine open state-dependently and benzocaine both open and closed state-dependently. We also conclude that a time and voltage-dependent block, similar to that reported for Na channels, with less inhibition of the peak current at high potentials than at low potentials, does not necessarily imply binding of channels in a closed state.

1112-Plat

Introducing Drug Action into Single-Cell Cardiac Models

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Drug development failures due to adverse cardiac effects cost the drugs industry millions of dollars every year. Many of these failures may be predicted through mathematical modelling of drug actions. In order to achieve this it is necessary to investigate the effectiveness of different ways of incorporating drug action into models. Five different single-cell cardiac models are studied with and without drug action. These comprise two rabbit models (Mahajan *et al.*, 2008; Shannon *et al.*, 2004) and three other species (ten Tusscher and Panfilov, 2006; Hinch *et al.*, 2004; Faber *et al.*, 2007). The L-type calcium channel regulation properties of the different models are compared, and their calcium-dependent and voltage-dependent inactivation properties are considered. It is found that the different models respond in very different ways to the introduction of drug action through a simple pore block with none of the models successfully reproducing experimental results for both drugs that are considered. It is therefore concluded that the kinetics of drug action on active and inactive channels must be included to better model the drug action. The differing responses of the models at different pacing frequencies and drug doses indicate that it is necessary to perform experiments at a range of frequencies and drug concentrations.

Platform S: Imaging & Optical Microscopy I

1113-Plat

Telomeres Diffusion Study Implies on A Self-Organization Mechanism of the Genome in the Nucleus

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The human genome contains tens of thousands of genes that are organized in chromosomes and packed in the nucleus of the cell. How can the chromosomes and DNA stay organized in territories without any compartmentalization? This order is sustained throughout the life cycle of a cell, a property that emerges as a key contributor to genome function, though its full extent is not yet known.

To address this question, we studied fluorescently-labeled telomeres diffusion in a broad time range of 10⁻² - 10⁴ seconds by combining a few microscopy methods followed by comprehensive diffusion analysis [1]. We found that the telomeres follow a complex diffusion pattern never reported before. The diffusion of the telomeres was found to be anomalous (subdiffusive) at short time scales and it changes to normal diffusion at longer times.

The transient diffusion indicates that telomeres are subject to a local binding mechanism with a wide but finite time distribution.

We therefore suggest that local temporal binding mechanism leads to the maintenance of structures and positions in the nucleus without the need for actual